# CHROMOSOMES OF SOUTH AMERICAN BUFONIDAE *(AMPHIBIA, ANURA)*

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The *Bufonidae* family is classified among the amphibia anura because of its degree of evolution, being considered as one of the most representative groups of the order Salientia.

We began the study of several species of the genus *Bufo* some years ago using the squash technique and we thought it of interest to report these results.

The family of South American *Bufonidae* has not been investigated much from the cytological point of view. Sáez, Rojas and De Robertis (1934[a], 1934[b], 1935, 1936), described the meiotic process in male *B. arenarum* and indicated that this species has 2n = 22 chromosomes. Sáez and Brum (1960), Bianchi and Laguens (1964), Ullerich (1967), Bogart (1967), Morescalchi (1968) and Brum-Zorrilla (1968) also found 2n = 22 chromosomes in different South American species of this genus.

## MATERIAL AND METHODS

The specimens examined and their source of origin are shown in Table I *. Small fragments of gonads from adult males were fixed in acetic-acid in a 3:1 ratio after pretreatment with NaCl hypotonic solution (0,5%) for 15 minutes. Two hours before sacrificing the female *B. arenarum* subjects 0.5 ml. of 0.05% colchicine solution was injected into the peritoneal cavity. Following sacrifice small spleen and intestine fragments were extracted and pretreated with distilled water, and squashed in acetichematoxylin and acetic-orcein. Identification and measurements were made from good spread metaphases previously drawn from a camera lucida. A centromeric index was obtained by the relation between the size of the short arm and the total length of the chromosome.

## RESULTS

In every species studied, 22 metacentric and submetacentric chromosomes were found grouped in six pairs of long chromosomes and five pairs of short chromosomes (Figs. 1 - 2).

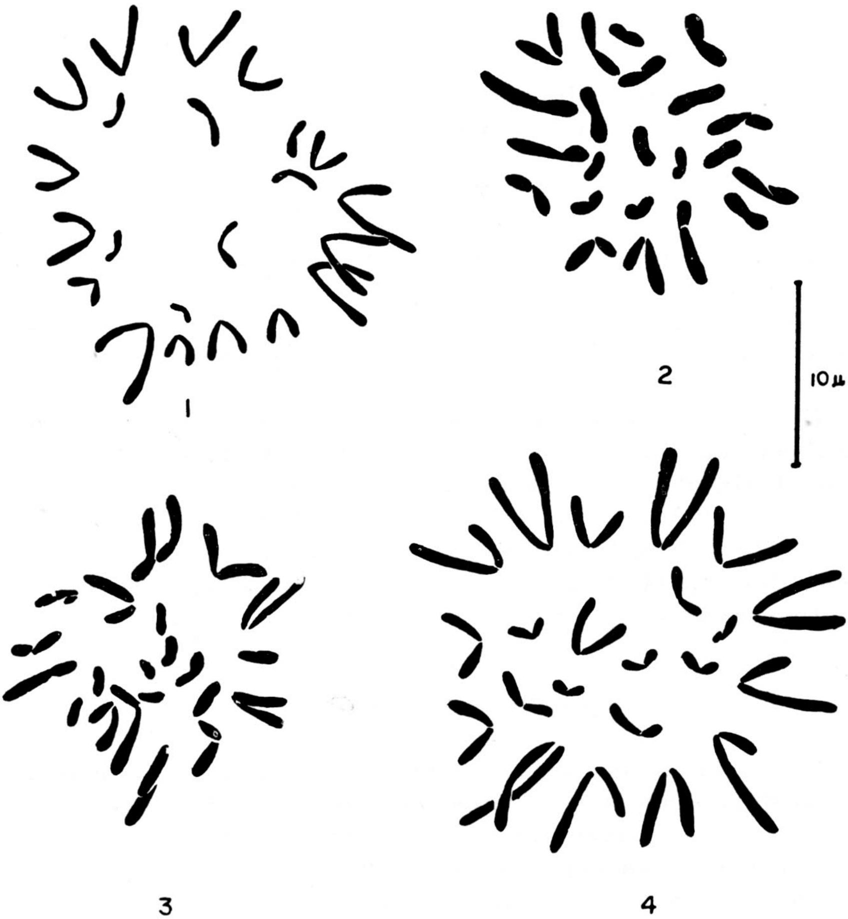

FIGURE 1. Metaphase of different species drawn with camera lucida. 1. *B. arenarum*. 2. *B. crucifer*, 3. *B. g. fernandezae* and 4. *B. ictericus*.

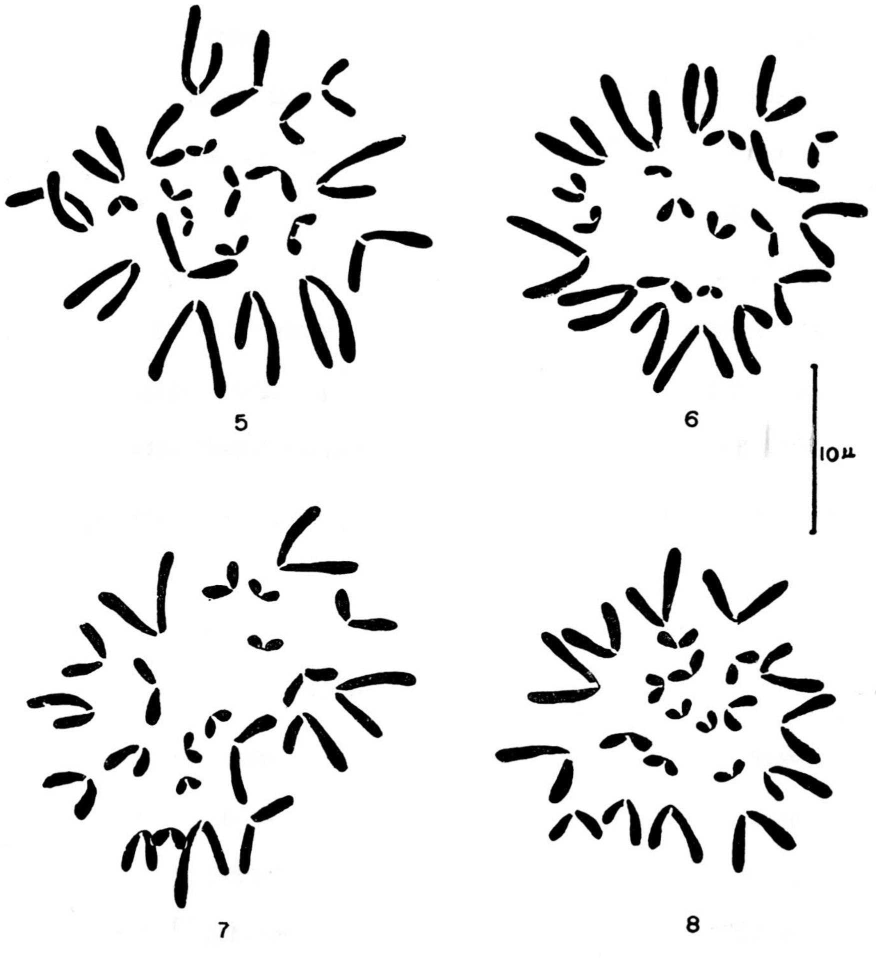

FIGURE 2. Metaphase of: 5. *B. spinulosus*, 6. *B. paracnemis*, 7. *B. marinus and* 8. *B. g. d'orbignyi.*

Figure 3 illustrates the idiograms of the different species. The chromosome pairs are arranged in decreasing of order size and identified on the basis of their size and centromeric position. The bivalent configuration is similar to that found in every amphibia anura and consists of the diplonema stage of a ring shaped element having two distal chiasmata. Even in the larger bivalents there are no interstitial chiasmata.

In Figure 4 the bivalents are arranged in decreasing order. At the beginning of metaphase I the bivalents lose their anular form and become

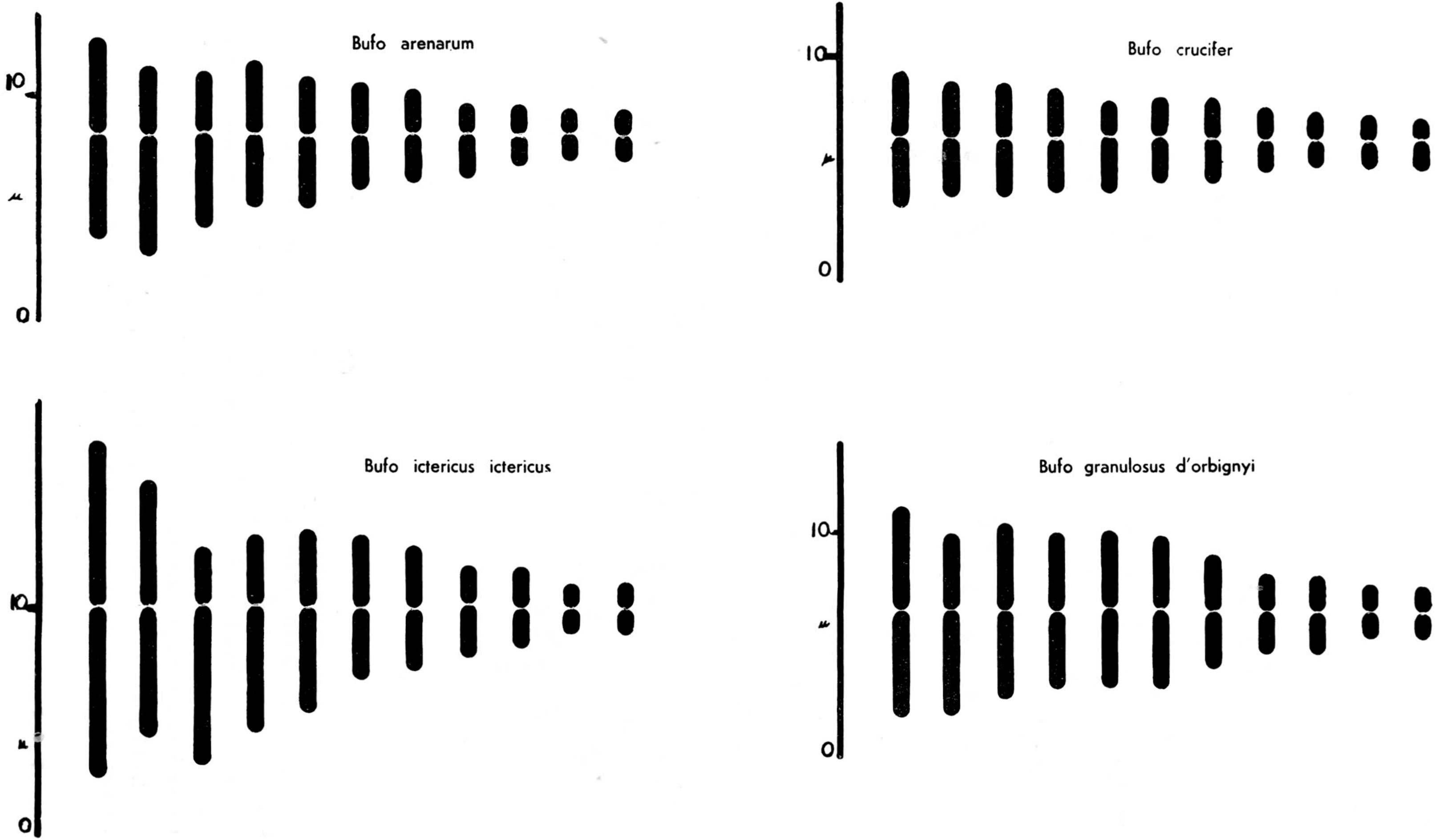
Bufo arenarum
10
μ
0
Bufo crucifer
10
μ
0
Bufo ictericus ictericus
10
μ
0
Bufo granulosus d'orbignyi
10
μ
0

FIGURE 3. Idiograms of species examined.

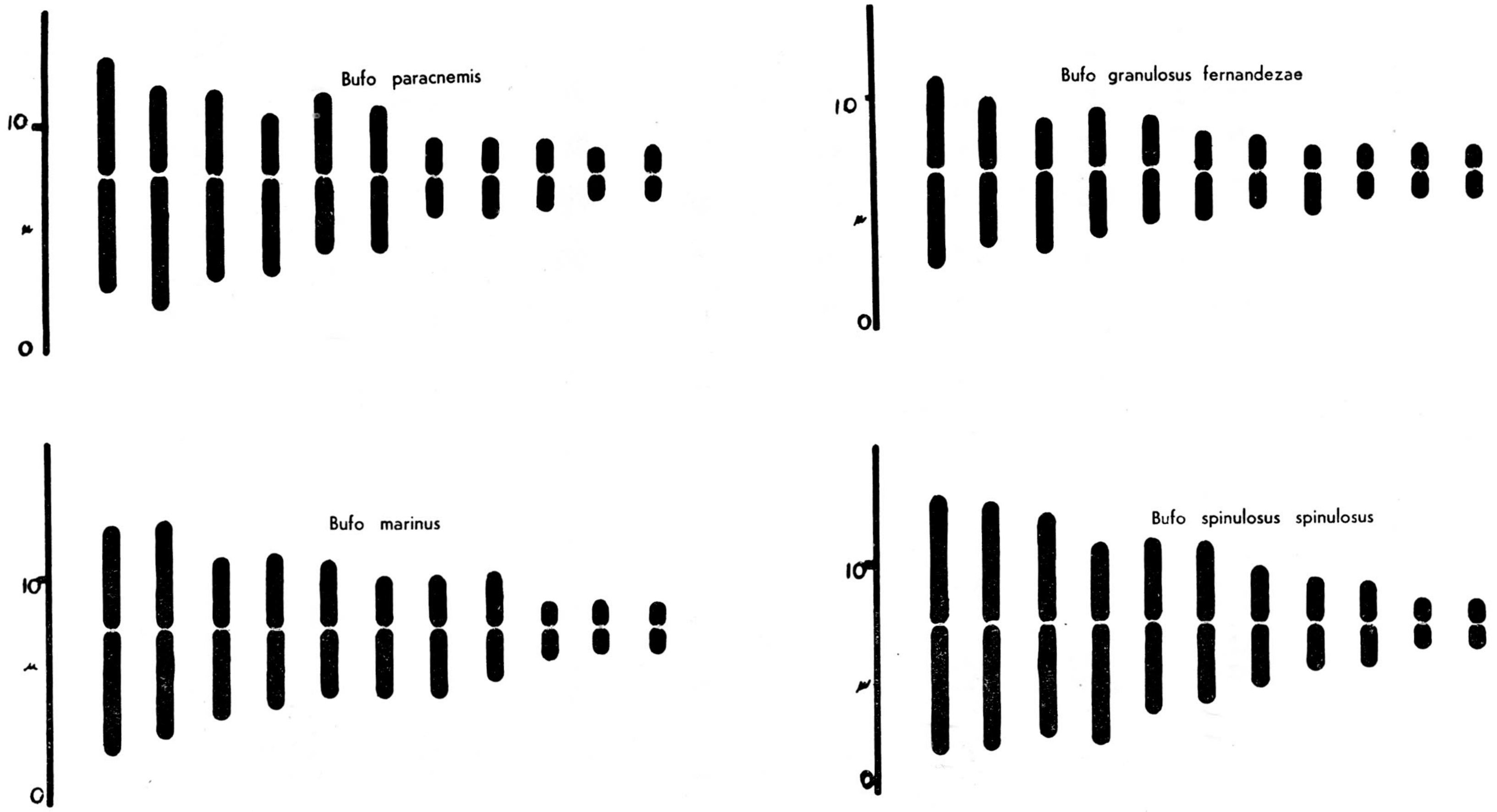
Bufo paracnemis
10
μ
0
Bufo granulosus fernandezae
10
μ
0
Bufo marinus
10
μ
0
Bufo spinulosus spinulosus
10
μ
0

FIGURE 3. Cont.

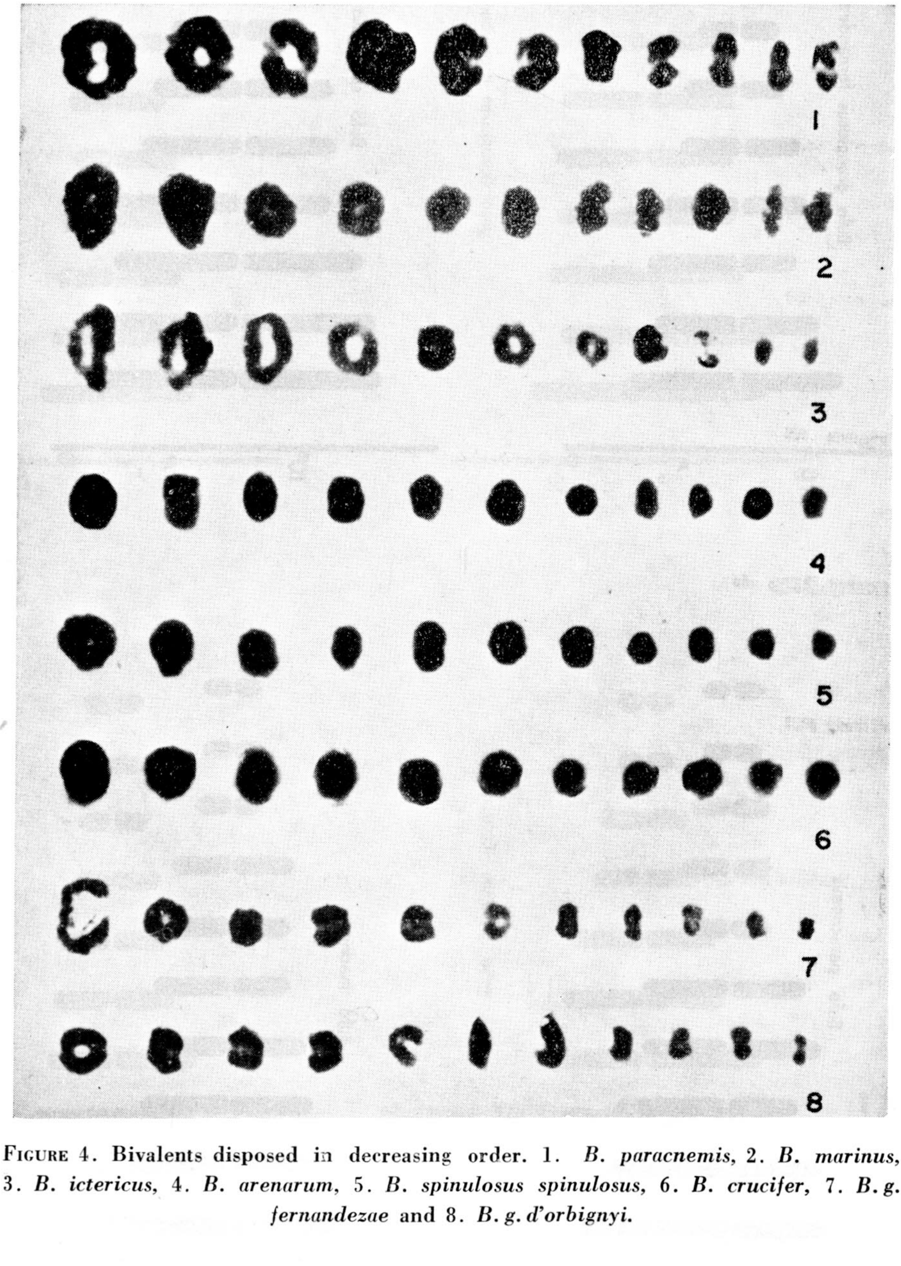

FIGURE 4. Bivalents disposed in decreasing order. 1. *B. paracnemis*, 2. *B. marinus*, 3. *B. ictericus*, 4. *B. arenarum*, 5. *B. spinulosus spinulosus*, 6. *B. crucifer*, 7. *B.g. fernandezae* and 8. *B.g. d'orbignyi.*

highly condensed compact elements whose structures are difficult to distinguish.

In all specimens of the different species, the meiotic process is similar to that studied and described by Sáez, Rojas and De Robertis (1936). Positive heteropicnotic chromosomes were not found in any stage of the meiotic prophase or in the first meiotic metaphase. It was not possible to see heterochromatic regions with the method we have employed. In the female *B. arenarum* secondary constrictions were found in the short arms of the homologous of the seventh pair (Fig. 5). (Brum-Zorrilla, 1968).

In Table II the values of the centromeric index obtained in the different species are tabulated. We were unable to find heteromorphic homologous pairs in any of the species.

## DISCUSSION

A diploid number of 2n = 22 chromosomes has been found for all of the species of the genus *Bufo* studied up to this time with the exception of those belonging to the *regularis* group from Africa in which Morescalchi (1968) found 2n = 20 chromosomes.

In the species studied here there was, as has been reported by others, generally a morphological uniformity. However Morescalchi (1968) found heterochromatic regions located in different parts of the chromosomes and in different pairs of homologues in several species of the genus *Bufo*. We were unable to observe those in our material, probably due to the technique we used. The existence of cytologically differentiated sex chromosomes is still disputable. Our investigations on amphibia anura fundamentally on South American amphibia started several years ago, have not demonstrated the presence of cytologically differentiated sex chromosomes in *Bufonidae* Sáez *et al.* 1934, 1935, 1936), *Ceratophrydae* (Sáez and Brum, 1960), *Leptodactylidae* (Brum-Zorrilla and Sáez, 1968) or *Hylidae* species (Brum-Zorrilla and Sáez, 1968).

Several authors have found heteromorphic pairs that have been labelled as sex chromosomes. Yosida (1957) described an XY pair in the male *Hyla arborea*, Weiler and Ohno (1962) found heteromorphism in female *Xenopus laevis*. Morescalchi (1964) described them in *Discoglosus pictus*, and more recently Manna and Bhunya (1966) reported heterogamety in the *B. melanostictus* female. Sáez *et al.* (1934, 1935, 1936) studied in detail the problem of the existence of the sex chromosomes in amphibia anura specifically in the *B. arenarum* species. They concluded that the presumed sex chromosomes found in different species of amphibia by other authors were only bivalents that had a different behavior. In this work it was pointed out that these bivalent elements and functions are nothing but common chromosomes which can have different shapes, size and affecting different chromosomes of the same individual.

Therefore we state that there are no sufficient data available yet to prove the existence of the sex chromosomes in spite of the presence of difference in the size of the members in a pair of homologues.

## SUMMARY

Karyotypes of eight of South American *Bufonidae* were studied: *B. ictericus, B. spinulosus spinulosus, B. arenarum, B. g. fernandezae, B. g. d'orbignyi, B. crucifer, B. paracnemis* and *B. marinus.* In all species 2n = 22 chromosomes were found. Neither heteromorphic pairs of chromosomes nor bivalents with characteristic morphology and behavior of sex chromosomes during male meiosis were observed in any species.

## ACKNOWLEDGEMENT

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### TABLE I

SPECIMENS EXAMINED AND SOURCE OF ORIGIN

| Species | Diploid number (2n) | Source |
|---|---|---|
| B. marinus .. .. .. .. .. .. | 22 | Paramaribo (Suriname) |
| B. paracnemis .. .. .. .. .. | 22 | Artigas (Uruguay) |
| B. ictericus .. .. .. .. .. .. | 22 | Sao Paulo (Brazil) |
| B. arenarum ♀ .. .. .. .. .. | 22 | Montevideo (Uruguay) |
| B. arenarum ♂ .. .. .. .. .. | 22 | Montevideo (Uruguay) |
| B. spinulosus spinulosus .. .. | 22 | La Paz (Bolivia) |
| B.g. fernandezae .. .. .. .. | 22 | Corrientes (Argentina) |
| B.g. d'orbignyi .. .. .. .. .. | 22 | Montevideo (Uruguay) |
| B. crucifer .. .. .. .. .. .. | 22 | Sao Paulo (Brazil) |

## TABLE II

## CENTROMERIC INDEX OF EIGHT SPECIES STUDIED

| Pair of chromosomes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B. marinus .. .. .. .. .. .. .. .. | .45 | .50 | .40 | .44 | .50 | .44 | .50 | .50 | .50 | .46 | .40 |
| B. paracnemis .. .. .. .. .. .. .. | .40 | .50 | .37 | .44 | .34 | .39 | .50 | .50 | .50 | .50 | .40 |
| B. ictericus .. .. .. .. .. .. .. .. | .50 | .50 | .36 | .37 | .43 | .50 | .40 | .50 | .45 | .50 | .50 |
| B. arenarum ♂ .. .. .. .. .. .. .. | .48 | .36 | .41 | .48 | .45 | .50 | .47 | .45 | .45 | .45 | .42 |
| B. arenarum ♀ (somatic chromosomes) .. .. .. .. .. .. .. .. | .47 | .39 | .42 | .48 | .44 | .48 | .45 | .44 | .45 | .44 | .43 |
| B. spinulosus spinulosus .. .. .. .. | .41 | .45 | .50 | .33 | .40 | .39 | .46 | .44 | .45 | .50 | .48 |
| B.g. d'orbignyi .. .. .. .. .. .. .. | .47 | .45 | .33 | .45 | .49 | .45 | .45 | .45 | .47 | .50 | .50 |
| B. crucifer .. .. .. .. .. .. .. .. | .46 | .50 | .34 | .50 | .47 | .50 | .50 | .40 | .50 | .48 | .47 |
| B.g. fernandezae .. .. .. .. .. .. | .46 | .49 | .33 | .46 | .40 | .46 | .45 | .45 | .45 | .50 | .45 |

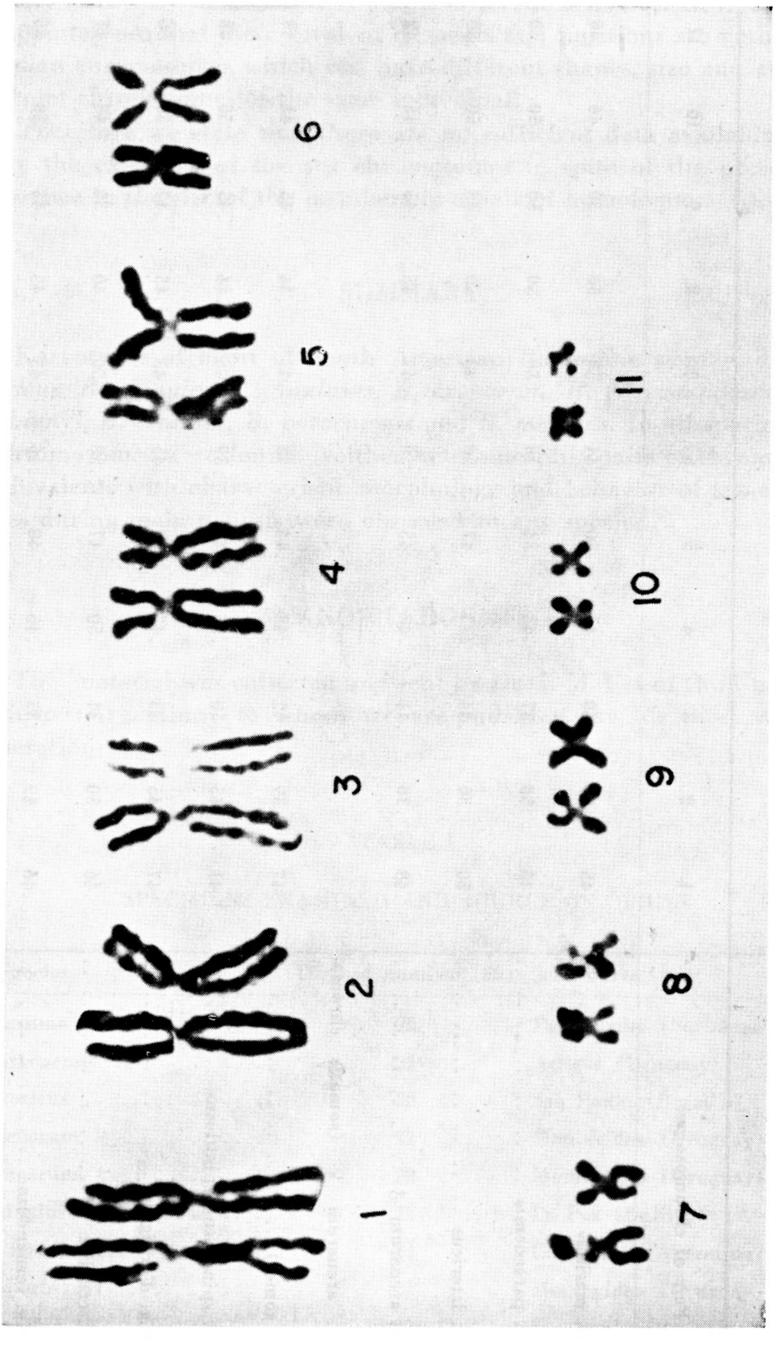

FIGURE 5. *B. arenarum* female karyotype from spleen cell. The 7th. pair shows one constriction on the homologous short arm.

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